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<p>(54) Title: HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE</p> <p>(57) Abstract</p> <p>The present invention is directed to imparting stress resistance to plants. This can be achieved by applying a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart stress resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart stress resistance to plants or plants grown from the plant seeds.</p>			

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HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

5

FIELD OF THE INVENTION

10 The present invention relates to imparting stress resistance to plants with a hypersensitive response elicitor.

BACKGROUND OF THE INVENTION

15 Under both natural and agricultural conditions, plants are exposed to various forms of environmental stress. Stress is mainly measured with respect to growth (i.e. biomass accumulation) or with respect to the primary assimilation processes (i.e. carbon dioxide and mineral intake). Soil water deficits, suboptimal and supraoptimal temperatures, salinity, and poor aeration of soils may each cause some 20 growth restrictions during the growing season, so that the yield of plants at the end of the season expresses only a small fraction of their genetic potential. Indeed, it is estimated that in the United States the yield of field-grown crops is only 22% of genetic potential. The same physicochemical factors can become extreme in some habitats, such as deserts or marshes, and only specially adapted vegetation can 25 complete its life cycle in the unusually hostile conditions. In less extreme environments, individual plants can become acclimated to changes in water potential, temperature, salinity, and oxygen deficiency so that their fitness for those environments improves. Some species are better able to adapt than others, and various anatomical, structural, and biochemical mechanisms account for acclimation.

30 Under natural and agriculture conditions, plants must constantly endure stress. Some environmental factors can become stressful in a very short period of time (e.g., high or low temperature) or may take long periods of time to stress plants (e.g., soil water content or mineral nutrients). Generally, environmental stress effecting plants can be in the form of climatic related stress, air pollution stress,

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chemical stress, and nutritional stress. Examples of climate related stress include drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light. Air pollution stress can be in the form of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain. Chemical stress can result from application of insecticides, fungicides, herbicides, and heavy metals. Nutritional stress can be caused by fertilizers, micronutrients, and macronutrients.

For most plants, water is essential for growth. Some plants are able to preserve some water in the soil for later use, while others complete their life cycles during a wet season before the onset of any drought. Other plants are able to aggressively consume water to save themselves while causing water deprivation for other plants in that location. Plants lacking any of these capabilities are severely hampered by the absence of water.

Chilling injury occurs in sensitive species at temperatures that are too low for normal growth but not sufficiently low to form ice. Such injury typically occurs in species of tropical or subtropical origin. When chilling occurs, discoloration or lesions appear on leaves giving them a water-soaked appearance. If roots are chilled, the plants may wilt. On the other hand, freezing temperatures and the accompanying formation of ice crystals in plants can be lethal if ice crystals extend into protoplasts or remain for long periods.

Stress is also caused by the other temperature extremes with few plants being able to survive high temperatures. When higher plant cells or tissues are dehydrated or are not growing, they can survive higher temperatures than cells which are hydrated, vegetative, and growing. Tissues which are actively growing can rarely survive at temperatures above 45°C.

High salt concentrations are another form of environmental stress which can afflict plants. In natural conditions, such high concentrations of salt are found close to seashores and estuaries. Farther inland, natural salt may seep from geological deposits adjoining agricultural areas. In addition, salt can accumulate in irrigation water when pure water is evaporated or transpired from soil. About 1/3 of all irrigated farmland is effected by high salt concentrations. High salt content not

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only injures plants but degrades soil structure by decreasing porosity and water permeability.

Air pollution in the form of ozone, carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, and hydrocarbons can very adversely effect plant growth by
5 creating smog and environmental warming.

The present invention is directed to overcoming various forms of environmental stress and imparting resistance in plants to such stress.

SUMMARY OF THE INVENTION

10 The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart
15 stress resistance. Alternatively, stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

20 Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Applicants have found that use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

30 **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one

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embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, the stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a 5 hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

The hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides 10 or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include 15 *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor 20 protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from 25 *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser 1 5 10 15	
Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser 20 25 30	
Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr 35 40 45	
Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu 50 55 60	

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Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
 65 70 75 80
 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
 85 90 95
 5 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
 100 105 110
 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
 115 120 125
 10 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
 130 135 140
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
 145 150 155 160
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
 165 170 175
 15 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
 180 185 190
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
 195 200 205
 20 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
 210 215 220
 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
 225 230 235 240
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
 245 250 255
 25 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
 260 265 270
 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
 275 280 285
 30 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
 290 295 300
 Asn Leu Asn Leu Arg Gly Ala Gly Ala Ser Leu Gly Ile Asp Ala
 305 310 315 320
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
 325 330 335
 35 Asn Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains

substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

5	CGATTTTACCGGGGTGAAACCGTGCTATGACC GACAGCATCA CGGTATTGCA CACCGTTACG GGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCCGCT GGTGCCGCA ATCCGGCGTC GATCTGGTAT TTCAAGTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCAGCAGAG TGCATGGCT GCCATCTGTC CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	60 120 180 240 300
10	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTGAACT GGCAGGAATG ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACCGGCC CGATCATTAA GATAAAGGGG GCTTTTTTA TTGCAAAACG GTACGGTGA GGAACCGTTT CACCGTCGGC GTCACTCACT AACAAAGTATC CATCATGATG CCTACATCGG GATCGCCGTG GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	360 420 480 540 600
15	AATTACGATC AAAGCGACA TCGGCGGTGA TTTGGCGTC TCCGGTCTGG GGCTGGGTGC TCAGGGACTG AAAGGACTGA ATTCCGCCG TTCATCGCTG GGTCCAGCG TGGATAAACT GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGCGCGCT GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGCCAGTC TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CGGGCGCGA	660 720 780 840 900
20	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCTG ACACCGTGAC CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAAACGCCA GCCAGATGAC CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGGCT TCTCTGGGG CAGGCGGCTT GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	960 1020 1080 1140 1200
25	GGGGCAGAAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA CGGCCACTTT GTAGATAAG AAGATCGGG CATGGCGAAA GAGATCGGCC AGTTTATGGA TCAGTATCCG GAAATATTG GTAAACCGGA ATACCAAGAAA GATGGCTGGA GTTCGCCGAA GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG CGCCAGCATG GACAAATTC GTCAGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1260 1320 1380 1440 1500
30	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACCCCTGATA	1560 1620

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	ATCTGTGCTG	GCCTGATAAA	GCGGAACGA	AAAAAGAGAC	GGGGAAGCCT	GTCTCTTTTC	1680
	TTATTATGCG	TTTATGCGG	TTACCTGGAC	CGGTTAACATCA	TCGTCATCGA	TCTGGTACAA	1740
	ACGCACATTT	TCCCGTTCAT	TCGCGTCGTT	ACCGGCCACA	ATCCGCGATGG	CATCTTCCTC	1800
	GTCGCTCAGA	TTGCGCGGCT	GATGGGGAAC	GCCGGGTGGA	ATATAGAGAA	ACTCGCCGGC	1860
5	CAGATGGAGA	CACGTCTGCG	ATAAAATCTGT	GCCGTAACGT	GTTCCTATCC	GCCCCCTTAG	1920
	CAGATAGATT	GGGGTTTCTG	AATCAACATG	GTAATGCGGT	TCCGCTGTG	CGCCGGCGG	1980
	GATCACCCACA	ATATTCTAG	AAAGCTGTCT	TGCACCTACC	GTATCGCGGG	AGATACCGAC	2040
	AAAATAGGGC	AGTTTTGCG	TGGTATCCGT	GGGGTGTCC	GGCCTGACAA	TCTTGAGITG	2100
	GTTCGTCATC	ATCTTCTCC	ATCTGGCGA	CCTGATCGGT	T		2141

10

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID.

No. 3 as follows:

15 Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
 1 5 10 15
 Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
 20 25 30
 20 Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn
 35 40 45
 25 Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
 50 55 60
 30 Met Met Met Ser Met Met Gly Gly Gly Leu Met Gly Gly Gly Leu
 65 70 75 80
 35 Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
 85 90 95
 40 Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
 100 105 110
 45 Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
 115 120 125
 50 Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
 130 135 140
 55 Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
 145 150 155 160
 60 Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175

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Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
180 185 190
Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
195 200 205
5 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
210 215 220
Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225 230 235 240
Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
10 245 250 255
Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
260 265 270
Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
275 280 285
15 Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
290 295 300
Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305 310 315 320
Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
20 325 330 335
Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
340 345 350
Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
355 360 365
25 Gly Asn Leu Gln Ala Arg Gly Ala Gly Ser Ser Leu Gly Ile Asp
370 375 380
Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385 390 395 400
Gly Ala Ala

30 This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from
35 *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

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Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTGAA TTATTCAAA	60
	GAGGAATAACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
	ATCGGGGGTG CGGGCGAAA TAACGGGTG CTGGGTACCA GTCGCCAGAA TGCTGGGTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCCGC GGTAATCAAAT ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGACCATGA TGGGCGGTGG TGGGCTGATG	300
10	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTGCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAAACACGCT GGGCTCGAAA	420
	GGCGGCAACA ATACCACTTC AACAAACAAT TCCCCCTGG ACCAGGCCTG GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTCAAGC GAGATAATGC AAAGCCTGTT TGTTGATGGG	600
15	CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCCCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGTA ATGCTGGCAC GGGCTTGAC	780
	GGTTCGTCGC TGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAAGC GCTGAATGAT	900
20	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGTCAGTT CATGGACAG TATCCGTGAGG TGTTGGCAA GCCGCAGTAC	1020
	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGCAGG AGCACTGAGC	1080
	AAGCCAGATG ACCACGGAAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
	ATGATCAAAT GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
25	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
	CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

Another potentially suitable hypersensitive response elicitor from
30 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927,
which is hereby incorporated by reference. The protein is encoded by a DNA
molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

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	ATGTCAATTG TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCGGG	60
5	GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT	120
	CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGAAC TGTTAAAGTC ACTGCTATCG	180
	CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT	240
10	AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT	300
	CAGAACATGC TGAGTGAGAT GGGCAACAAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC	360
15	CAGGGCGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA	420
	CCCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC	480
	TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTAACG ATCTATCAGG GGGGAAGGCC	540
20	CCTTCGGCA ACTCCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCATCC	600
	ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTCTT CTCCCCACCAA AGCAGCCGGG	660
25	GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGGGGCAT CGGGGCCGGA	720
	AATTGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGATGAA CACCATTAACC	780
	GTCAGGGCGG GTCAGGTGTT TGATGGCAA GGACAAACCT TCACCGCCGG TTCAAGAATTAA	840
30	GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTA TACTGGAAGA CGGTGCCAGC	900
	CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC	960
35	AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC	1020
	AGCGCGGCCA AAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC	1080
	AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC	1140
40	TTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC	1200
	CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC	1260
	GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAACACC ACTACAAAGT GCCGATGTCC	1320
45	GCCAACCTGA AGGTGGCTGA ATGA	1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present
50 invention encodes a hypersensitive response elicitor protein or polypeptide having an
amino acid sequence of SEQ. ID. No. 6 as follows:

55	Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu	
	1 5 10 15	
	Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser	
	20 25 30	
60	Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala	
	35 40 45	

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Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
50 55 60

5 Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65 70 75 80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
85 90 95

10 Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
100 105 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp
15 115 120 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
130 135 140

20 Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145 150 155 160

Ser Ser Gly Thr Ser Ser Ser Gly Ser Pro Phe Asn Asp Leu Ser
165 170 175

25 Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
180 185 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
30 195 200 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
210 215 220

35 Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225 230 235 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
245 250 255

40 Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
260 265 270

45 Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
275 280 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
290 295 300

50 Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305 310 315 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
325 330 335

55 Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
340 345 350

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	Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
	355 360 365
5	Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
	370 375 380
	Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
	385 390 395 400
10	His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
	405 410 415
	Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
	420 425 430
15	Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
	435 440 445

20 This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from
25 *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

30	ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAAC	60
	CCTGTGGGGC ATGGTGTTC CTTACAGCAG GGCACAGCA GCAGCAGCCC GCAAAATGCC	120
	GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGAAAAA TGCCGAGAAAT TCACCCAGCA	180
35	TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG	240
	GGCTGTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGTACCCACC	300
40	CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGG ACGACGGCGA AACGCAGCAT	360
	GAGGCGGCCG CGCCAGATGC GGCGCGTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT	420
	ATGGACGACA TGGCCGGGGC GCCAATGGTG AAAGGTGGCA CGGGCGAAGA TAAGGTACCA	480
45	ACGCAGCAAA AACGGCATCA GCTGAACAAT TTGGCCAGA TGGGCCAAC GATGTTGAGC	540
	AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATGCC TGCAGCATTC ACCGCCGAC	600
50	ATCCCGGTA GCCACCACCA AATCAAGGAA GAACCGGTTG GCTCCACCAAG CAAGGCAACA	660
	ACGGCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA	720
	CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC	780
55	GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC	840

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	CTTGAGGGGA CAGATACCAAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT	900
5	GGAGCCGGGG TAACGCCGCT GGCGGTAAACG CTGGATAAAAG GCAAGTTGCA GCTGGCACCG	960
	GATAATCCAC CGCGCCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC	1020
	TATCTGGCGC ACCATGCCAG CAGCGACCGT ACCCAGCATC TGCTGCTGGA CAACAAAGGC	1080
10	CACCTGTTG ATATCAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC	1140
	GGTGAGATAA AGGGCAAGCT GGCGCAGCGG GGTACTGGCT CCGTCAGCGT AGACGGTAAA	1200
	ACCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAAGCCAA	1260
15	CGGGGGGAAG CGCACCGTTC CTTATTAAAC GGCAATTGGC AGCATCCTGC TGGCGCAGCG	1320
	CGGCCGCAGG GCGAGTCAT CGCCTGCAT GACGACAAAA TTCATATCCT GCATCOGGAG	1380
20	CTGGCGTAT GGCAATCTGC GGATAAAAGAT ACCCACAGCC AGCTGCTCG CCAGGCAGAC	1440
	GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAA ACCTCTCCGA TAATAATCC	1500
	TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG	1560
25	ATCCTGACGG ATACTCCCGG CGCCATAAG ATGAGTATTG TGCCCTCGCT GGATGCTTCC	1620
	CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTGCGGATG CCCACCAAGGG GTTATTGCAC	1680
30	GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGCGACT GGTTGTGGCC	1740
	GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG	1800
	AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAAGATT	1860
35	TCTGGATTTC TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTAAAAAA TAACCTCAGG	1920
	CAGCAGCATG CCTGCCCTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT	1980
40	GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG	2040
	ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACATTTT	2100
	GACCAGCTGA CCAAAGGGT GACTGGCCCG GAGTCAGATT GTAAGCAGCT GAAAAAAAGGC	2160
45	CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCTGAA TATTAATCAG	2220
	AGCACCTCCT CTATCAAGCA CGGAACCGAA AACGTTTTTG CGCTGCCGCA TGTGCGCAAT	2280
50	AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG	2340
	GCGGTGATTG GGGTAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC	2400
	CAGATAAAAC CGGGCACCCA GCAGTTGGAG CGGCCGGCAC AAGCTCTCAG CCGCGAAGGT	2460
55	ATCAGCGCGC AACTGAAAGA CATTGATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC	2520
	CACGAGGGAG AGGTGTTCA TCAGCCGGT GAAGCCTGGC AGAACCTGTC CGAAAGCAGC	2580
60	AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC	2640
	CATGAGCACA AACCGATTGC CACCTTGAA GACGGTAGCC AGCATCAGCT GAAGGGCTGGC	2700
	GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA	2760
65		

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	CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG	2820
	TTGACCGTTA AGCTCTCGGC TCAGACGGGG GGAAATGACCG GCGCCGAAGG GCCAAGGTC	2880
5	AGCAGTAAAT TTTCCGAAAG GATCCCGGCC TATGCGTTCA ACCAACAAAT GTCCACGCCG	2940
	CGACCGATTAA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG	3000
10	AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAACAAAC TGGATGCGCA TAACGTTCGT	3060
	CATAACCGCGC CACAGCCAGA TTTGCAGAGC AAACCTGGAAA CTCTGGATTT AGGCGAACAT	3120
	GGCGCAGAAT TSCCTAACCA CATGAAGCGC TTCCCGACG AACTGGACCA GAGTGCAACC	3180
15	CGTTCGGTGA CCGTTTTAGG TCAACATCG AGGAGTGCTAA AAAGCAACGG TGAAATCAAT	3240
	AGCGAATTAA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT	3300
20	GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG	3360
	AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCAG GGGTGGATAT GAGTCATCAG	3420
	AAGGGCGAGA TCCCCTGGG CGGCCAGCGC GATCCGAATG ATAAAACCCG ACTGACCAAA	3480
25	TCGCGTTAA TTTTAGATAC CGTGACCATC GGTGAACATGC ATGAACCTGGC CGATAAGGCG	3540
	AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCC CCAGCAGTTC	3600
30	GATACTGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC	3660
	TTCACCCATA ATAAGGCAGCT GGAAGCRAAC TATGATGCCGG TCAAAGCCTT TATCAATGCC	3720
	TTTAAGAAAG AGCACCAACCG CGTCANTCTG ACCACGCTA CCGTACTGGA ATCACAGGGC	3780
35	AGTGCAGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT	3840
	ATGAGCTTCA GCCGGTCATA TGGCGGGGC GTCAGCACTG TCTTGTGCC TACCTTACG	3900
40	AAGAAGGTGC CAGTTCCGGT GATCCCCGGA CGCCGCATCA CGCTGGATCG CGCCTATAAC	3960
	CTGAGCTTCA GTCGTACCAAG CGGCGGATTG AACGTCAGTT TTGGCCGCCA CGGGGGGTG	4020
	AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA	4080
45	ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG	4140
	CGTATCGGGC CTGCTGTGAG TGGCACCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT	4200
50	AAGCTGACAG AGGATGAGCT GCCTGGTTT ATCCATGGCT TGACGCATGG CACGTTGACC	4260
	CGGGCAGAAC TGGTGAAAGA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG	4320
	TTTACCGTGC ATACCTCGGC AAATCTGGAT CTGCGTGCG GTATCAATCT GAACGAAGAC	4380
55	GGCAGTAAAC CAAATGGTGT CACTGCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAAC	4440
	CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGCC AGTTTGGCAG CACGACTTCG	4500
60	GCCAGCAATA ACCGCCAAC CTTCCCTAAC GGGGTGGCG CGGGTGCTAA CCTGACGGCT	4560
	GCTTTAGGGG TTGCCCCATTC ATCTACCGCAT GAAGGGAAAC CGGTGGGAT CTTCCCGCA	4620
	TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC	4680
65	AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC	4740

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	ACGCTGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTGCCGC TCTCAAAGAG	4800
5	TTAGATGACG CTAAGCCCC TGAAACAATG CATACTTAC AGCAGCATTT CAGTGCAAAA	4860
	GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATAACGT	4920
	CAACAGGCTG CGGACAGCCA CAGCATGAA TTAGGATCTG CCAGTCACAG CAOGACCTAC	4980
10	AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTCGAT	5040
	GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA	5100
15	CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTGATG	5160
	GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAGCAA TACTGGACGG TAAGGTCGGT	5220
	CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAAACAAT TCCGTGTAA ATCGGTCAAGC	5280
20	GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG	5340
	AGCAACACGG CTGCTATGAG CATGGAGCGC AACATCGAA CCATTAATTT TAAATACGGC	5400
25	CAGGATCAGA ACACCCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG	5460
	CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGCG TGAAATGAA GAGCTAA	5517

This DNA molecule is known as the *dspE* gene for *Erwinia amylovora*. This isolated
 30 DNA molecule of the present invention encodes a protein or polypeptide which elicits
 a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.
 ID. No. 8 as follows:

35	Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr	
	1 5 10 15	
	Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser	
	20 25 30	
40	Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly	
	35 40 45	
	Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala	
	50 55 60	
45	Asp Gly Ile Ser Ala Ala His Gln Gln Lys Ser Phe Ser Leu Arg	
	65 70 75 80	
50	Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln	
	85 90 95	
	Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Ala	
	100 105 110	
55	Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala	
	115 120 125	
	Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met	
	130 135 140	
60		

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	Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro	
	145 150 155 160	
5	Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln	
	165 170 175	
	Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp	
	180 185 190	
10	Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile	
	195 200 205	
	Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala	
	210 215 220	
15	Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln	
	225 230 235 240	
20	Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro	
	245 250 255	
	Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys	
	260 265 270	
25	Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln	
	275 280 285	
	Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val	
	290 295 300	
30	Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro	
	305 310 315 320	
35	Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys	
	325 330 335	
	Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln	
	340 345 350	
40	His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr	
	355 360 365	
	Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys	
	370 375 380	
45	Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys	
	385 390 395 400	
50	Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr	
	405 410 415	
	Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile	
	420 425 430	
55	Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg	
	435 440 445	
	Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp	
	450 455 460	
60	Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp	
	465 470 475 480	
65	Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser	
	485 490 495	

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	Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser			
	500	505	510	
5	Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg			
	515	520	525	
	His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His			
	530	535	540	
10	Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His			
	545	550	555	560
	Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg			
	565	570	575	
15	Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro			
	580	585	590	
20	Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His			
	595	600	605	
	Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe			
	610	615	620	
25	His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg			
	625	630	635	640
	Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly			
	645	650	655	
30	Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His			
	660	665	670	
35	His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly			
	675	680	685	
	Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr			
	690	695	700	
40	Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly			
	705	710	715	720
	Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu			
	725	730	735	
45	Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val			
	740	745	750	
50	Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu			
	755	760	765	
	Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly			
	770	775	780	
55	Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe			
	785	790	795	800
	Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu			
	805	810	815	
60	Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His			
	820	825	830	
65	Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln			
	835	840	845	

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	Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys			
	850	855	860	
5	Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser			
	865	870	875	880
	His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln			
	885	890	895	
10	Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro			
	900	905	910	
	Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met			
	915	920	925	
15	Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys			
	930	935	940	
20	Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val			
	945	950	955	960
	Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr			
	965	970	975	
25	Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His			
	980	985	990	
	Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly			
	995	1000	1005	
30	Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro			
	1010	1015	1020	
	Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His			
35	1025	1030	1035	1040
	Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu			
	1045	1050	1055	
40	Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val			
	1060	1065	1070	
	Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly			
	1075	1080	1085	
45	Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu			
	1090	1095	1100	
	Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu			
50	1105	1110	1115	1120
	Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp			
	1125	1130	1135	
55	Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro			
	1140	1145	1150	
	Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val			
	1155	1160	1165	
60	Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser			
	1170	1175	1180	
65	Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe			
	1185	1190	1195	1200

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	Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr			
	1205	1210	1215	
5	Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp			
	1220	1225	1230	
	Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val			
	1235	1240	1245	
10	Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu			
	1250	1255	1260	
15	Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser			
	1265	1270	1275	1280
	Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val			
	1285	1290	1295	
20	Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly			
	1300	1305	1310	
	Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly			
	1315	1320	1325	
25	Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile			
	1330	1335	1340	
30	Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys			
	1345	1350	1355	1360
	Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile			
	1365	1370	1375	
35	Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly			
	1380	1385	1390	
	Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro			
	1395	1400	1405	
40	Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu			
	1410	1415	1420	
45	Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr			
	1425	1430	1435	1440
	Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn			
	1445	1450	1455	
50	Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser			
	1460	1465	1470	
	Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg			
	1475	1480	1485	
55	Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn			
	1490	1495	1500	
60	Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala			
	1505	1510	1515	1520
	Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly			
	1525	1530	1535	

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	Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu			
	1540	1545	1550	
5	Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu			
	1555	1560	1565	
	Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys			
	1570	1575	1580	
10	His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu			
	1585	1590	1595	1600
	Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His			
	1605	1610	1615	
15	Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg			
	1620	1625	1630	
20	Asn Leu Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser			
	1635	1640	1645	
	Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser			
	1650	1655	1660	
25	Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp			
	1665	1670	1675	1680
	Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn			
	1685	1690	1695	
30	Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro			
	1700	1705	1710	
35	Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu			
	1715	1720	1725	
	Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val			
	1730	1735	1740	
40	Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser			
	1745	1750	1755	1760
	Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu			
	1765	1770	1775	
45	Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile			
	1780	1785	1790	
50	Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg			
	1795	1800	1805	
	Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser			
	1810	1815	1820	
55	Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser			
	1825	1830	1835	

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

60 The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

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	ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTACAGT ATTTCTCCGC CGGGTGTAAA	60
5	ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAAGA TGAGGAGGCG	120
	GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG	180
	GCTGACCCAC AAACCTCAAT AACCCCTGTAT TCGATGCTAT TACAGCTGAA TTTGAAATG	240
10	GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACCTGC ACAACGTGCG TTTATGTTT	300
	CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTAGCG ATATCGTTAG CGGCTTCATC	360
	GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA	420

This is known as the *dspF* gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

20	Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
	1 5 10 15
25	Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
	20 25 30
30	Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
	35 40 45
35	Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
	50 55 60
40	Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
	65 70 75 80
45	Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
	85 90 95
50	Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
	100 105 110
55	Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
	115 120 125
60	Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
	130 135

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

50 The hypersensitive response elicitor polypeptide or protein derived
from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID.
No. 11 as follows:

55 Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1 5 10 15

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Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20 25 30

5 Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val
65 70 75 80

10 Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

15 Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

20 Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

25 Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

30 Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
35 275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

- 23 -

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325 330 335

Asn Gln Ala Ala Ala
340

- This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin_{Ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

15	ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCTG	60
	GTACGTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
	GTGAAGCTGG CGCAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
	AAAATGTTGG CCAAGTCGAT GGCGCGAGAT GGCAAGGGGG GCGGCGGTAT TGAGGATGTC	240
20	ATCGCTGGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCCTCTGCG	300
	GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCTGGCC	360
	AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC	420
	GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCGC ACAGTTTCCC	480
	AAGCCGGA CCGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC	540
25	GAAACGGCTG CGTTCGGTTC GGCACTCGAC ATCATGGCC AGCAACTGGG TAATCAGCAG	600
	AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTCC	660
	AACAACCTGT CGGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC	720
	GGCAATAACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCTGCAA	780
	TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAACCA CCCCGCAGAC CGGTACGTCG	840
30	GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG	900
	GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	960
	GCGCAAATCG CCACCTTGCT GGTCACTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA	1020
	GCCTGA	1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of
 5 SEQ. ID. No. 13 as follows:

	TCCACTTCGC	TGATTTTGA	ATTGGCAGAT	TCATAGAAC	GTTCAGGTGT	GGAAATCAGG	60
10	CTGAGTGC	GC AGATTTCG	TT GATAAGGGTG	TGGTACTGGT	CATTGTTGGT	CATTCAAGG	120
	CCTCTGAGTG	CGGTGCGGAG	CAATACCA	GT CTTCTGCTG	GC GTGTGCAC	ACTGAGTCGC	180
	AGGCATAGGC	ATTTCA	TTTCAGTTTC	CTTGCCTTGG	TTGGGCATAT	AAAAAAAGGA	ACTTTAAAAA
15	ACAGTGCAAT	GAGATGCCGG	CAAACGGGA	ACCGGTGCT	GCGCTTTGCC	ACTCACTTCG	240
	AGCAAGCTCA	ACCCCAAACA	TCCACATCCC	TATCGAACGG	ACAGCGATAC	GGCCACTTGC	300
20	TCTGGTAAAC	CCTGGAGCTG	GC GTGGTCC	AATTGCCCAC	TTAGCGAGGT	AACGCAGCAT	360
	GAGCATCGGC	ATCACACCCC	GGCCGCAACA	GACCACCA	CG CACTCGATT	TTTCGGCGCT	420
	AAGCGGCAAG	AGTCC	TCAC	CAAACACGTT	CGGCAGACAG	AAACACTCAGC	480
25	CCCGAGTGCA	CTGTTGTTCG	GCAGCGACAC	ACAGAAAGAC	GTCAACTTCG	GCACGCCCCGA	540
	CAGCACCGTC	CAGAATCCCG	AGGACGCCAG	CAAGCCAAAC	GACAGCCAGT	CCAACATCGC	600
	TAAATTGATC	AGTGCATTGA	TCATGTCGTT	GCTGCAGATG	CTCACCAACT	CCAATAAAA	660
30	GCAGGACACC	AATCAGGAAC	AGCCTGATAG	CCAGGCTCCT	TTCCAGAAC	ACGGCGGGCT	720
	CGGTACACCG	TCGGCCGATA	GC GGGGGCGG	CGGTACACCG	GATGCGACAG	GTGGCGGGCG	780
35	CGGTGATACG	CCAAGCGCAA	CAGGCGGTGG	CGGCGGTGAT	ACTCCGACCG	CAACAGGCGG	840
	TGGCGGCAGC	GGTGGCGGGCG	GCACACCCAC	TGCAACAGGT	GGCGCAGCC	GTGGCACACC	900
40	CACTGCAACA	GGCGGTGGCG	AGGGTGGCGT	AAACACGCAA	ATCACTCCGC	AGTTGGCCAA	960
	CCCTAACCGT	ACCTCAGGTA	CTGGCTCGGT	GTCGGACACC	GCAGGTTCTA	CCGAGCAAGC	1020
	CGGCAAGATC	AATGTTGTA	AAAGACACCAT	CAAGGTGCGC	GCTGGCGAAG	TCTTGTGACGG	1080
45	CCACGGCGCA	ACCTTCACTG	CCGACAAATC	TATGGTAAC	GGAGACCAGG	GCGAAAATCA	1140
	GAAGCCCATG	TTCGAGCTGG	CTGAAGGCC	TACGTTGAAG	AATGTAACC	TGGGTGAGAA	1200
	CGAGGTCGAT	GGCATCCACG	TGAAAGCCAA	AAACGCTCAG	GAAGTCACCA	TTGACAAACGT	1260
50	GCATGCCAG	AACTCGGTG	AAGACCTGAT	TACGGTCAA	GGCGAGGGAG	GCGCAGCGGT	1320
	CACTAACATCG	AAACATCAAGA	ACAGCAGTG	CAAAGGTGCA	GACGACAGG	TTGTCCAGCT	1380
55	CAACGCCAAC	ACTCACTTGA	AAATCGACAA	CTTCAAGGCC	GACGATTTCG	GCACGATGGT	1440
	TCGCACCAAC	GGTGGCAAGC	AGTTTGATGA	CATGAGCATC	GAGCTGAACG	GCATCGAAC	1500
60	TAACCACGGC	AAAGTTCGCC	TGGTGAAAG	CGACAGTGAC	GATCTGAAGC	TGGCAACGGG	1560

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CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA 1680
 CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC 1729

5

This DNA molecule is known as the *dspE* gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

10

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
 1 5 10 15

15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
20 25 30

20

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
50 55 60

25

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65 70 75 80

30

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
85 90 95

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
115 120 125

35

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
 130 135 140

40

Pro Ser Ala Thr Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145 150 155 160

4

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Ser Val
165 170 175

12

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
180 185 190

50

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
220 225 230

56

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Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
 245 250 255

5 Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
 260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
 275 280 285

10 Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
 290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Ala Ala
 305 310 315 320

15 Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
 325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
 20 340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
 355 360 365

25 Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
 370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
 385 390 395 400

30 Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
 405 410 415

Ala Ser Thr Gln His Thr Glu Leu
 35 420

This protein or polypeptide is about 42.9 kDa.

40 The hypersensitive response elicitor polypeptide or protein derived
 from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ.
 ID. No. 15 as follows:

45 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
 20 25 30

val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45

50 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60

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Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85 90 95

5 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
115 120 125

10 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly
165 170 175

15 Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly
180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala
195 200 205

20 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
245 250 255

25 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
275 280 285

30 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305 310 315 320

val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
325 330 335

35 Gln Ser Thr Ser Thr Gln Pro Met
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ.

ID. No. 16 as follows:

- 28 -

	ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
	AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTCC AAGACCTGAT CAAGCAGGTC	120
	GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCCGGCGGGC	180
	GGCAACACCG GTAACACCGG CAACCGCCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
5	AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
	GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
	GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCCG CAATGACAAG	420
	GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC	480
	GAAGCGCTGC AGGAGATCGA GCAGATCCCTC GCCCAGCTCG GCGGGCGCG TGCTGGCGCC	540
10	GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGTT	600
	GGGCCAGGCG GTGCGAACGG CGCCGACGGC GGCATATGGCG TGAAACGGCAA CCAGGGCGAAC	660
	GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC	720
	CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG	780
	ATGATGCAGC AAGGCGGCCT CGGCGGCCGC ACCAGGGCGC AGGGCGGCTC GAAGGGTGCC	840
15	GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CGGGCGCGA ACCAGCCCGG TTCGGCGGAT	900
	GATCAATCGT CGGGCCAGAA CAATCTSCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC	960
	GTCCAGATCC TGCAGCGAT GCTGGCGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG	1020
	ACGCAGCCGA TGTAA	1035

20 Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted
25 via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

30 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

5 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from
10 *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

15 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15
Leu Leu Ala Met
20

20 Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp N_{Ecc}* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," *MPMI*, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or
25 polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," *8th Int'l. Cong. Molec. Plant-Microbe Interact.*, July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," *Ann. Mtg. Am. Phytopath. Soc.*, July 27-31, 1996, which are hereby incorporated by reference.

30 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens,"
35 *Molec. Plant-Microbe Interact.*, 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and

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Acquired Resistance in Tobacco," *Eur. J. Biochem.*, 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," *Plant Path.* 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A

5 Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," *Plant J.*, 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," *Eur. J. Plant Path.*, 102:181-92 (1996), which are hereby incorporated by reference.

10 Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. *sepedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

15 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

20 Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

25 Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely 30 to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for 5 expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by 10 conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia*. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or 15 an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 105 and 179, 137 and 166, 121 and 150, or 20 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N- 25 terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 30 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the

following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under 5 stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml *E. coli* DNA.

Variants may be made by, for example, the deletion or addition of 10 amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, 15 purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the 20 present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove 25 bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

30 The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;

microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the 5 host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor 10 which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not 15 function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short 20 nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see 25 Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, 30 expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promoter,

trp promotor, *recA* promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promotor or other *E. coli* 5 promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted 10 DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation 15 initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to 20 provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD- 25 ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell 30 system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention's method of imparting stress resistance to plants can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart stress resistance. Alternatively, the hypersensitive response 5 elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart stress resistance in plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart stress resistance in plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized.

- 10 When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart stress resistance to plants. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor 15 polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart stress resistance to plants.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be 20 carried out in a number of ways, including: 1) application of an isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with genes encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding a hypersensitive response elicitor polypeptide 25 or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seed cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide 30 variety of plants or their seeds to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea,

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chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable 5 ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

In accordance with the present invention, the term "stress" refers to drought, salt, cold temperatures (e.g., frost), chemical treatment (e.g., insecticides, fungicides, herbicides, fertilizers), water, excessive light, and insufficient light.

10 The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable 15 application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, 20 coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After 25 plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants.

The hypersensitive response elicitor polypeptide or protein, in 30 accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof.

10 Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

15 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

20 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA.

25 Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

30 Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby

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incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles 5 with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other 10 entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by 15 electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

20 Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a 25 suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are 30 induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a

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convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the

gene encoding the hypersensitive response elicitor resulting in stress resistance to the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are 5 propagated from the planted transgenic seeds under conditions effective to impart stress resistance to plants. While not wishing to be bound by theory, such stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the 10 present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, 15 including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, 20 etc.).

EXAMPLES

25 **Example 1 - Hypersensitive Response Elicitor-Treated Cotton is More Resistant to the Damage Caused by Insecticide Stress**

Aphids (*Aphids gossypii*) infect cotton during the entire growth season. The damage of aphid infection ranges from honeydew deposit that contaminates the lint and reduces crop value to defoliation that reduces or destroys crops. To protect 30 plants from aphid infection, cotton is usually sprayed with insecticides, for example Asana XL when the infection pressure is not very high, and Admire when the infestation pressure is high. The effect of a hypersensitive response elicitor on aphids in cotton was studied by a trial involving a randomized complete block design. This

involved treatment with *Erwinia amylovora* hypersensitive response elicitor (i.e. HP-1000™) at 20, 60, and 80 ppm and a chemical insecticide, Asana XL, at 8 oz./ac. Each treatment involved foliar application beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a backpack sprayer. Aphid counts and overall growth of the cotton were made immediately prior to spray application at 14, 28, 35, and 42 days after the first treatment ("DAT 1"). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates and the leaves per plot at the final sampling date.

10 Results

1. Aphid control: The number of aphids in the hypersensitive response elicitor-treated cotton were significantly reduced in comparison to the chemical treated cotton (see Table 1).

15 Table 1. Aphid count per leaf on cotton after treatment with Asana XL® or HP-1000™

Treatment	Rate ²	Number of aphids per leaf ¹ No. sprays applied/days after treatment			
		1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL®	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000™	20 µg/ml	0.2 a	7.8 b	22.9 b	322.1 a
HP-1000™	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000™	80 µg/ml	0.0 a	2.7 b	25.8 b	510.2 a

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. ²Rate for Asana XL® is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

20 At 14 days after DAT 1, aphid counts were relatively low across all of the treatments, but by 28 days after DAT 1 (by which time two sprayings had been applied), the number of aphids per leaf were significantly greater in Asana XL-treated plants compared to the hypersensitive response elicitor-treated cottons. By 35 days after DAT 1 (by which time three sprayings had been applied), aphid counts had risen for all treatments, yet aphid counts per leaf were still significantly lower for hypersensitive response elicitor-treated cotton compared to the Asana XL treatment. 30 Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the number of aphids per leaf had increased to a level that threatened to overwhelm the

plants even when treated with the standard chemical insecticide. To save the trial, another chemical, Pravado (Admire), was applied to all plots to eradicate aphids from the field.

2. Hypersensitive response elicitor-treated cotton was more
5 resistant to the damage caused by Pravado (Admire) and Asana. After the second
chemical spraying, it was observed that cotton plants were stress shocked by the
insecticides. The cotton plants previously treated with Asana and untreated control
were defoliated. On most of the chemical-treated cotton, there were no leaves, or
very few leaves, in the lower portion of plants. However, the hypersensitive response
10 elicitor-treated plants, especially the plot where hypersensitive response elicitor was
applied at 80 ppm, had no defoliation and the cotton plants were vigorous and healthy.
By counting the number of mature balls, it clearly showed that hypersensitive
response elicitor-treated plants (at 80 ppm) had more ball setting than chemical and
untreated control (Table 2), indicating that hypersensitive response elicitor-treated
15 plants were more tolerant to the stress caused by insecticide.

Table 2. Number of Formed Cotton Balls Counted on Ten Plants
in Each of Four Replicates Per Treatment.

20	<u>Treatment</u>	<u>No. balls/10 plants/replicate</u>
	UTC	28
	Chemical standard	6
25	Hypersensitive Response Elicitor	35

Example 2 - Hypersensitive Response Elicitor-Treated Cucumbers are More Resistant to Drought

30 A cucumber field trial was set up to test the effect of *Erwinia amylovora* hypersensitive response elicitor on disease control, tolerance to drought stress, and yield. Three different rates were tested, there at 15, 30, and 60 µg/ml. In addition to hypersensitive response elicitor treatment, there was an untreated control. Each treatment contained three replicate plots. When the first true leaf emerges,
35 hypersensitive response elicitor was sprayed with a back bag sprayer. The second spray was applied ten days after the first spray. The third application was right after

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the recovery of cucumber seedlings after the transplanting to the field. Individual treatment was randomly assigned in the field.

When the first true leaf emerged (Day 0), a first application was sprayed. Usually cucumber seedlings are transplanted when seedlings show two true leaves. It has been known that the recovery rate after the transplanting is closely related to the size of the seedlings. Because of the drought, the seedlings were maintained in the nursery for an extra ten days and the second spray was applied on Day 10. Two days after the second spray, the plants were transplanted into fields and covered with plastic sheets. The plants had 4 – 5 true leaves.

10

Result

The recovery rate of the transplanted cucumber seedlings was higher for the hypersensitive response elicitor-treated plants than for the untreated control. More than 80% of the hypersensitive response elicitor-treated cucumber seedlings 15 survived, while only 57% untreated plants survived.

Throughout the growth season, there was a serious drought problem. Early field visits indicated that hypersensitive response elicitor-treated plants had more root mass and better over-all growth. Hypersensitive response elicitor-treated cucumber started to flower 14 days earlier than untreated control cucumber. The 20 early flowering resulted in an earlier harvest. In the first harvest, more than 0.4 kilograms of cucumber fruits per plant were harvested from the hypersensitive response elicitor-treated cucumbers; however, virtually no fruit was harvested from untreated control. By the end of the season, untreated plants died due to severe drought, but hypersensitive response elicitor-treated plants were still alive and had 25 one more harvest.

The final yield was significantly different between hypersensitive response elicitor-treated and untreated plants. Hypersensitive response elicitor administered at the rate of 30 ppm produced three times greater yield than the control plants (Table 3).

30

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Table 3. Yield Increase of Cucumber Fruit from Hypersensitive Response Elicitor Treated Plants

Treatment	Replicate	kg/plant	Yield/Replicate	% of the Yield Increase
HP 15	I	1.25	37.5	
	II	1.00	30.0	103.8
	III	1.21	36.3	
HP 30	I	1.54	46.2	
	II	1.43	42.9	133.2
	III	1.47	44.1	
Control	I	0.43	12.9	
	II	0.41	12.3	39.3
	III	0.47	14.1	

5

The increased yield was partially attributed to hypersensitive response elicitor-induced growth enhancement and partially resulted from more tolerance of hypersensitive response elicitor-treated cucumber to drought, because usually the yield increase from hypersensitive response elicitor-induced growth enhancement is
10 between 10-40%.

10

Example 3 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress

15 Pepper seedlings were drenched with hypersensitive response elicitor at 20 ppm seven days before transplanting, sprayed seven days after the transplanting, and then, sprayed every fourteen days. Standard chemicals, Brave, Maneb, Kocide, and Admire, were used for the rest of the treatment. In addition to early growth enhancement, which resulted in a higher yield, larger fruit, and resistance to several
20 diseases, hypersensitive response elicitor-treated pepper was more tolerant to herbicide damage. The pepper field was applied with the herbicide SENCOR which is not labeled for pepper. This herbicide is known to cause severe foliar damage to pepper in chemically-treated plants but not with hypersensitive response elicitor-treated plants.

25 The difference between the adverse effect of the herbicide on the hypersensitive response elicitor and non-hypersensitive response elicitor treated plants is dramatic. See Table 4 below. Thirty-nine of the 60 elicitor-treated plants showed only minor damage by the herbicide, the damaged leaves were less than 20%. In

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contrast, 53 out of the 60 chemically-treated pepper plants had severe damage, 40-57% of the leaves were damaged, and 20 plants were dead. The ability of hypersensitive response elicitors to help crops withstand the phytotoxic effects of a herbicide is very important benefit to in agricultural industry.

5

Table 4. Hypersensitive Response Elicitor-Treated Peppers are More Tolerant to Herbicide Damage.

	<u>Treatment</u>	<u>Damage Rating</u>						<u>Damage Index %</u>
		1	2	3	4	5	6	
10	Hypersensitive Response Elicitor	1	38	17	3	1	0	41
15	Chemicals	0	1	6	16	19	18	87

Damage Rating: 1. No damage; 2. 0-20% leaves damaged; 3. 20-40% leaves damaged; 4. 40-50% leaves damaged; 6. More than 75% leaves damaged or entire plant dead.

20

Damage index = sum of each rating times the number of plants under the rating scale, divided by total number of plants times 6.

Damage index for hypersensitive response elicitor-treated plants =

$$\frac{1 \times 1 + 2 \times 38 + 3 \times 17 + 4 \times 3 + 5 \times 1 + 6 \times 0}{6} \times 100\% = 41\%$$

25

Example 4 - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress under Controlled Experimental Conditions

30

A field trial was conducted to test if hypersensitive elicitor treated pepper would be more tolerant to herbicide stress. The trial contains 6 treatments and 4 replicates for each treatment. The treatments are described as follows:

1. Control, the peppers were neither treated by a hypersensitive response ("HR") elicitor nor by LEXONE™ herbicide (DuPont Agricultural Products, Wilmington, Delaware).
2. Control pepper with application of 0.15 pound LEXONE™ herbicide /acre.
3. Control pepper with application of 0.3 pound LEXONE™ herbicide /acre.

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4. HR elicitor treatment with no application of LEXONE™ herbicide using a formulated product known as MESSENGER™ biopesticide (Eden Bioscience Corporation, Bothell, Washington) containing 3% HR elicitor protein was used.
5. HR elicitor treatment with application of 0.15 pound LEXONE™ herbicide /acre.
 6. HR elicitor treatment with application of 0.3 pound LEXONE™ herbicide /acre.

LEXONE™ contains the same active ingredient as SENCORT™

10 herbicide (Bayer, Kansas City, Missouri) used in Example 3. Pepper seedlings were drenched with MESSENGER™ solution at the concentration of HR elicitor protein of about 20 ppm seven days before transplanting into the field and then sprayed every 14 days after the transplanting. LEXONE was applied at high (0.3 pound/acre) and low levels (0.15 pound/acre). 50 gallon water and 100 mL of the herbicide solution was

15 introduced into the root zone of each plant in the respective treatment five weeks after transplant into the field.

The treatments were evaluated for the percent of chlorosis caused by the LEXONE™ herbicide application and for the pepper yield. HR elicitor-treated plants exposed to the high rate of herbicide had significantly less chlorosis and

20 produced 108 % more fruit in comparison to the non-hypersensitive response elicitor treated plants exposed to the same amount of herbicide. See Tables 5 and 6 below. There was no significant difference in the reduction of chlorosis at the low rate of herbicide between the HR elicitor treated and non-HR elicitor treated peppers.

However, the HR elicitor treated plants produced 15% more fruit than the

25 corresponding control plants exposed to the same amount of herbicide. There was no chlorosis in either the check or HR elicitor-treated plants that did not receive LEXONE™ herbicide treatment.

The HR elicitor treated plants were much less severely affected by the herbicide application than the respective control plants at the high rate of herbicide.

30 However, the amount of visual chlorosis was similar at the low rate for both the check and HR elicitor-treated plants. More importantly, the yields from both the high and low rate herbicide treatments of HR elicitor treated plants were less severely effected

by the herbicide than the checks. These findings further confirm that HR elicitors can help crops withstand the phytotoxic effects of herbicides and are very beneficial to the agricultural industry.

5 Table 5. Reduction of Foliar Chlorosis and Increase in Yield in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide

Treatment	Percent foliar chlorosis and yield of pepper					% difference from the respective control	
	A	B	C	D	E		
6 (MESSENGER™ + High rate LEXONE™)	13.75	30.00	37.50	36.25	40.00	8.31	108 %
3 (High rate LEXONE™)	26.25	43.75	51.25	50.00	51.25	4.00	-
5 (MESSENGER™ + low rate LENOXE™)	16.25	22.50	28.75	23.75	27.50	8.00	15 %
2 (LENOXE™)	12.50	20.00	25.00	25.00	23.75	6.81	-

10 Table 6. Weight of Harvested Peppers Increased in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide Compared to Check Plants.

Treatment	Weight of peppers harvested 12/1/98 in pounds
HP20 + high rate LEXONE™	8.31
Check + high rate LEXONE™	4.00
HP20 + low rate LEXONE™	8.00
Check + low rate LEXONE™	6.81

15 Example 5 - Hypersensitive Response Elicitor-Treated Cotton is More Tolerant to Drought Stress

16 A non-irrigated cotton trial experienced 26 consecutive days of drought. The average daily heat index was near or over 100 degrees F, adding to the 20 stress placed on the plants in the field.

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- Observations in the field indicated that plants treated with HR elicitor at the concentration of 15 ppm (2.2 oz formulated product, MESSENGER™ containing 3 % active ingredient HR elicitor protein) were more vigorous and had less defoliation than the check plants as a result of the heat and drought stress. Equal 5 numbers of plants from the MESSENGER™-treated and the non-MESSENGER™ treated plots were carefully removed from the field and mapped for the number of nodes and bolls by position. The plants were also weighed on a Metler analytical scale to determine whole plant, root and shoot weights.
- MESSENGER™ treated plants survived the heat and drought stresses 10 much better than the untreated plants did. Plants treated with MESSENGER™ had 37.6% more root and shoot mass than the check plants (Table 7). The MESSENGER™ treated plants also had significantly more cotton bolls than the check plants (Table 8). The number of cotton bolls from positions 1 and 2 have a significant contribution to the overall yield. Table 8 showed that MESSENGER™ 15 treated plants had 47% more bolls in positions 1 and 2 and 57% more boll from a whole plant in comparison to the yield achieved using a grower standard treatment (i.e. with no MESSENGER™ treatment). A common reaction to stress in cotton is for the plant to abort bolls. The results indicate that MESSENGER™-treated plants are more tolerant to the drought stress.
- 20

Table 7. Weight per Plant of Non-Irrigated Cotton Following 26 Days of Drought.

Treatment	Root weight (pond/plant)	%Difference	Shoot weight (pond/plant)	% difference	Whole plant weight (pond/plant)	% difference
MESSENGER™ 2.2 oz/acre	0.041 a*	37.6 %	0.505 a	37.5 %	0.546	37.5 %
Control (Grower standard)	0.0298 b		0.367 b		0.397	
Level of statistically significant	P=0.119		P=0.034			P=0.033

25

* Same letter indicates no statistical difference between the two treatments at the defined level; different letter indicates a statistical difference between the two treatments at the defined level.

Table 8. Number of Bolls per 5 Plants at the Number 1 & 2 positions, and Total Number of Bolls from Whole Plants in Non-irrigated Cotton Following 26 days of drought.

5

Treatment	Avg. # bolls in the #1 & 2 position	Percent difference	Avg. # of total bolls per 5 plant	Percent difference
MESSENGER™ 2.2 OZ	18.4 a	+46.0%	21.4 a	+57.0%
Check	12.6 b		13.6 b	-
Statistically significant level	P=0.032		P=0.01	

* Same letter indicates no statistical difference between the two treatments at the defined level;
different letter indicates a statistical difference between the two treatments at the defined level.

10 **Example 6 - Hypersensitive Response Elicitor-Treated Tomato is More Tolerant to Calcium Deficiency**

15 Calcium is an important element for plant physiology and development. A deficiency in calcium can cause several plant diseases. For example, blossom-end rot is caused by a localized calcium deficiency in the distal end of the tomato fruit. Because calcium is not a highly mobile element, a deficiency can occur with a fluctuation in water supply. In the past, tomato growers experienced higher level of blossom-end rot during dry weather conditions when infrequent rains storms dumped a lot of water and then return to a hot and dry condition quickly. Lowering or 20 raising the irrigation water table erratically during a dry and hot growing season can also increase the disease.

25 A field trial was designed to test if HR elicitor protein-treated tomato can be more tolerant to the calcium deficiency under a dry hot growing season. MESSENGER™, the formulated product containing 3% HR elicitor, was used for the trial. The application rate of the MESSENGER™ was 2.27 oz per acre. The first spray of MESSENGER™ was carried out 7 days before the transplanting and then every 14-days after transplanting. MESSENGER™-treated tomatoes were compared with a standard grower treatment not utilizing MESSENGER™. Each treatment had 4 replicates.

30 The number of infected fruit was counted from a 100 square foot field. The rot typically begins with light tan water soaked lesions, which then enlarge, and then turn black. In a survey, about 20% of the fruits were infected. Severe end-rot

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symptoms occurred in the standard treatment; however, an average of only 2.5 % of the fruit was infected in the MESSENGER™-treated plants. The harvest data showed that MESSENGER™-treated plants had 8% more marketable fruit (Table 9). The test results demonstrated that MESSENGER™-treatment can reduce the stress resulting
5 from calcium deficiency and increase plant resistance to blossom-end rot.

Table 9. Hypersensitive Response Elicitor Treatment Reduced Blossom-End Rot Infection, Increased Yield of Tomato Fruit

10

Treatment	Blossom-End Infected Fruit*				Tomato Fruit Yield	
	Rep I	Rep II	Rep III	Rep IV	Bin/Acre	% Difference
MESSENGER™	0	9	0	1	35	8
Standard Treatment)	24	22	16	17	31.5	-

*The data were collected from the fruits in 100 square foot plot

Although the invention has been described in detail for the purpose of
15 illustration, it is understood that such detail is solely for that purpose, and variations
can be made therein by those skilled in the art without departing from the spirit and
scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of imparting stress resistance to plants comprising:
applying a hypersensitive response elicitor protein or
5 polypeptide in a non-infectious form to a plant or plant seed under conditions
effective to impart stress resistance.
2. A method according to claim 1, wherein the stress resistance is
resistance to a stress selected from the group consisting of climated related stress, air
10 pollution stress, chemical stress, and nutritional stress.
3. A method according to claim 2, wherein the stress is chemical
stress where the chemical is selected from the group consisting of insecticides,
fungicides, herbicides, and heavy metals.
15
4. A method according to claim 2, wherein the stress is climate-
related stress selected from the group consisting of drought, water, frost, cold
temperature, high temperature, excessive light, and insufficient light.
- 20 5. A method according to claim 2, wherein the stress is air
pollution stress selected from the group consisting of carbon dioxide, carbon
monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic
rain.
- 25 6. A method according to claim 2, wherein the stress is nutritional
stress where the nutritional stress is caused by fertilizer, micronutrients, or
macronutrients.
- 30 7. A method according to claim 1, wherein the hypersensitive
response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*,
Xanthomonas, *Phytophthora*, or *Clavibacter*.

8. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.

5 9. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or
Pseudomonas solancearum.

10 10. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.

11. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Phytophthora*.

15 12. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Clavibacter michiganensis* subsp. *sepedonicus*.

13. A method according to claim 1, wherein plants are treated
20 during said applying.

14. A method according to claim 1, wherein plant seeds are treated during said applying, said method further comprising:
planting the seeds treated with the hypersensitive response
25 elicitor protein or polypeptide in natural or artificial soil and
propagating plants from seeds planted in soil.

15. A method according to claim 1, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,
30 peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

16. A method according to claim 1, wherein the plant is selected
5 from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

17. A method of imparting stress resistance to plants comprising:
providing a transgenic plant or plant seed transformed with a
10 DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and
growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

15 18. A method according to claim 17, wherein a transgenic plant is provided.

19. A method according to claim 17, wherein a transgenic plant seed is provided, said method further comprising:
20 planting the transgenic seeds in natural or artificial soil and propagating plants from seeds planted in soil..

20. A method according to claim 17, wherein the stress resistance is resistance to a stress selected from the group consisting of climated related stress,
25 air pollution stress, chemical stress, and nutritional stress.

21. A method according to claim 20, wherein the stress is chcmical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.

- 55 -

22. A method according to claim 20, wherein the stress is climate-related stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.

5 23. A method according to claim 20, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.

10 24. A method according to claim 20, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.

15 25. A method according to claim 20, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, or *Clavibacter*.

20 26. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Erwinia amylovora*, *Erwinia carotovora*, *Erwinia chrysanthemi*, and *Erwinia stewartii*.

25 27. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.

28. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from a *Xanthomonas* species.

29. A method according to claim 20, wherein the plant is selected
30 from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,

eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pincapple, soybean, tobacco, tomato, sorghum, and sugarcane.

- 5 30. A method according to claim 20, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

SEQUENCE LISTING

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RESISTANCE

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<210> 8

<211> 1838

<212> PRT

<213> Erwinia amylovora

<400> 8

Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr

1

5

10

15

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20

25

30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
35 40 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
50 55 60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65 70 75 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
85 90 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
100 105 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
115 120 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
130 135 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145 150 155 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
165 170 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
180 185 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
195 200 205

Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
210 215 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
225 230 235 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
245 250 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
260 265 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
275 280 285

Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
290 295 300

Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
305 310 315 320

Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
325 330 335

Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
340 345 350

His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
355 360 365

Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
370 375 380

Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
385 390 395 400

Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
405 410 415

Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
420 425 430

Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
435 440 445

Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
450 455 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
465 470 475 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
485 490 495

Asp Asn Lys Ser Ser Gln Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
500 505 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
515 520 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
530 535 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
545 550 555 560
Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
565 570 575
Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
580 585 590
Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
595 600 605
Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
610 615 620
His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625 630 635 640
Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
645 650 655
Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
660 665 670
His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
675 680 685
Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
690 695 700
Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
705 710 715 720
Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
725 730 735
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740 745 750
Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
755 760 765
Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
770 775 780
Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
785 790 795 800

Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
805 810 815

Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
820 825 830

Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
835 840 845

Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
850 855 860

Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
865 870 875 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
885 890 895

Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
900 905 910

Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
915 920 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
930 935 940

Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
945 950 955 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
965 970 975

Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
980 985 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
995 1000 1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
1010 1015 1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
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Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
1045 1050 1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
1060 1065 1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
1075 1080 1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
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Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105 1110 1115 1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
1125 1130 1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
1140 1145 1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
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Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
1170 1175 1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
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Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
1205 1210 1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
1220 1225 1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
1235 1240 1245

Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
1250 1255 1260

Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265 1270 1275 1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Val Ser Thr Val Phe Val
1285 1290 1295

Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
1300 1305 1310

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Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
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Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
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Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
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Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
 1365 1370 1375

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
 1380 1385 1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
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Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
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Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
 1425 1430 1435 1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
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Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
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Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
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Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
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Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
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Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
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Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
 1555 1560 1565

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Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
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 His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
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 Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
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 Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
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 1650 1655 1660
 Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
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 Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
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 Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
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 Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Va
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 Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Se
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 Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Le
 1765 1770 1775
 Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Il
 1780 1785 1790
 Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg A
 1795 1800 1805
 Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala S
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Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
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 <213> Erwinia amylovora

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 gctgacccac aaacttcaat aaccctgtat tcgatgttat tacagctgaa ttttggaaatg 240
 gcgcccatgc gcggctgttg gctggcgctg gatgaactgc acaacgtgcg ttatgtttt 300
 cagcagtgcg tggagcatct ggatgaagca agtttttagcg atatcgtag cggttcatc 360
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 <213> Erwinia amylovora

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Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
 35 40 45

Ser Asp Ser Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
 50 55 60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
 65 70 75 80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
 85 90 95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
 100 105 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
 115 120 125

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Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
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<210> 11
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<212> PRT
<213> *Pseudomonas syringae*

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Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
 35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
 50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val
 65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
 85 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
 100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
 115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
 130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
 145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
 165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
 180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly

195 200 205
Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val
260 265 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala
290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
325 330 335

Asn Gln Ala Ala Ala
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<210> 12

<211> 1026

<212> DNA

<213> *Pseudomonas syringae*

<400> 12

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gtgaagctgg ccgaggaact gatgcgcatt ggtcaactcg acgacagctc gccattggga 180
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WO 00/28055

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 gcctga

<210> 13

<211> 1729

<212> DNA

<213> Pseudomonas syringae

<400> 13

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 cctctgatgt cggtcgccgg caataccagt cttectgtg gctgtgcac acttgatgc 180
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<210> 14

<211> 424

<212> PRT

<213> Pseudomonas syringae

<400> 14
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Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
20 25 30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
35 40 45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
50 55 60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65 70 75 80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
85 90 95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
100 105 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
115 120 125

Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr
130 135 140

Pro Ser Ala Thr Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145 150 155 160

Gly Gly Ser Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
165 170 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Glu Gly Gly Val Thr
180 185 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
195 200 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
210 215 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225 230 235 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
260 265 270

Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
275 280 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
290 295 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Ala Ala
305 310 315 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
325 330 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
340 345 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
355 360 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
370 375 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385 390 395 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
405 410 415

Ala Ser Thr Gln His Thr Glu Leu
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<210> 15

<211> 344

<212> PRT

<213> *Pseudomonas solanacearum*

<400> 15

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1 5 10 15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
20 25 30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile

35

40

45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
50 55 60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65 70 75 80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
85 90 95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
100 105 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
115 120 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
130 135 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145 150 155 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly
165 170 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly
180 185 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala
195 200 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
210 215 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225 230 235 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
245 250 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
260 265 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
275 280 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser

290 **295** **300**

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met
340

<210> 16
<211> 1035
<212> DNA
<213> *Pseudomonas solanacearum*

<400> 16
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 gagaaggaca tcctcaacat catcgccagcc ctcgtgcaga agggccgacca gtcggcgccc 180
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 ggcggcgccgg gtggccgtgt cggccgtgt ggtggccggg atggccgctc cggtgccgg 600
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 gatcaatgtt cccggccagaa caatctgcgaa tcccagatca tggatgtgtt gaaggagggtc 960
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 acgcaggccgatgtaa 1035

<210> 17
<211> 26
<212> PRT
<213> *Xanthomonas campestris* pv. *glycines*

<400> 17
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1 5 10 15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

<210> 18

<211> 20

<212> PRT

<213> Xanthomonas campestris pv. pelargonii

<400> 18

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15

Leu Leu Ala Met

20